REMARKS

Claims 2, 4-5, 7-12 and 20 are under prosecution with the entry of this Amendment. Claims 13-19 have been canceled without prejudice as non-elected claims. Claim 2 has been amended to correct the dependency. Claim 3 has been canceled without prejudice. Claim 4 has been amended for improved clarity. Claim 20 has been amended to better claim the subject matter that Applicants regard as the claimed invention and for improved clarity. The Specification has been amended to be consistent with the drawings submitted herewith. None of the amendments made herein constitutes the addition of new matter.

Drawings:

Formal drawings are included with this response together with a Transmittal of Formal Drawings.

Claim Rejection under 35 USC § 112:

Claims 2-5 and 7-12 remain rejected under 35 USC § 112, first paragraph, with the Patent Office alleging that the Specification, while being enabling for a method of identifying the metastatic potential of a cancer cell line that expresses the gene encoding MUC18, does not reasonably provide enablement for a method of identifying the metastatic potential of any prostate cancer cell. Applicant respectfully traverses this rejection.

With the entry of this Amendment, amended claim 20 specifically recites that the claimed invention is for predicting an increased risk for metastasis of a prostate cancer cell expressing a MUC18 coding sequence by measuring the levels of MUC18 expression. If a given prostate cancer cell does not express the MUC18 coding sequence, there is no basis for predicting the risk of metastasis by the claimed invention. Accordingly, the claimed invention is not for predicting an increased risk for metastasis of any prostate cancer cell, but rather for predicting increased risk where the MUC18 sequence is expressed.

In order to demonstrate that the claimed invention can indeed be used with a reasonable expectation of success to predict an increased risk for metastasis of a prostate cancer cell, submitted herewith is a Declaration of Guang-Jer Wu under 37 C.F.R. 1.132.

As illustrated in the Declaration, five different types of samples were examined for human MUC18 expression. These include 28 normal epithelium, 11 benign prostatic hyperplasia, 31 prostatic intraepithelial neoplasia (PIN), 32 carcinoma, and 5 metastatic carcinoma. As shown in Table 1, human MUC18 polypeptide was essentially undetectable in the normal and benign prostatic hyperplasia samples whereas the immunochemical staining in the high grade, pre-malignant PIN, malignant prostate carcinoma, and metastatic carcinoma was positive in more than 80% of the specimens. A majority of the metastatic carcinoma samples were stained strongly. These results clearly provide a positive correlation between the levels of MUC18 expression and different stages of malignancy, i.e., the level of MUC18 expression is increased progressively as the cells change from normal and BPH, pre-malignant, malignant, to metastatic carcinoma.

Further illustrated in the Declaration are the studies that confirm the basis for using the expression levels of MUC18 as a diagnostic marker for predicting an increased risk of a prostate cancer cell. In these studies, a cell line, LNCaP, that does not express MUC18 and is known to be non-metastatic, was transfected to express human MUC18 polypeptide. The three cell lines stably expressing human MUC18 at high levels showed increased cell motility and invasiveness compared to the control, untransfected LNCaP cells *in vitro* (see Figure 1). The measurements of cell motility and invasiveness are the standard assays indicative of metastatic ability of a cancer cell. Furthermore, when the cells expressing human MUC18 were orthotopically injected into the prostate glands of male nude mice, these animals showed increased tumor-take of the LNCaP cells in the prostate gland as well as the metastasis of the cells from the prostate gland to a number of tissues including the seminal vesicles, the kidney, and the peri-aortic lymph nodes. In

contrast, animals received the control cells (i.e. parental LNCaP cells) did not show these phenotypes.

In summary, the studies discussed above confirm the fact that MUC18 plays a critical role in the metastatic process and that the level of MUC18 expression can be a predictor of an increased risk for metastasis of a prostate cancer cell as claimed in the present application.

Based on the foregoing, it is respectfully submitted that one skilled in the art would have been able to practice the claimed invention without the expense of undue experimentation based on the teachings of the Specification, taken with what is well know in the art. Withdrawal of the rejection under 35 U.S.C. § 112, first paragraph, is respectfully requested.

Rejection under 35 U.S.C. § 112, second paragraph:

Claims 2-5, 7-12, and 20 are rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite. This rejection is based on the usage of the term, "metastatic potential" therein. Applicant respectfully traverses this rejection.

Without acquiescing to this rejection and in the interest of advancing the prosecution of this application, claim 20 has been amended and no longer recites the term. It is believed that amended claim 20 defines the invention with improved clarity. Accordingly, withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, is respectfully requested.

Rejection under 35 U.S.C. § 102:

Claims 2-5 and 20 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Rubenstein *et al.*, Shih *et al.*, Liu *et al.*, and the annotation in the Swiss protein Database. Applicant respectfully traverses this rejection.

The purpose of the Rubenstein *et al.*'s study was to demonstrate the potential applicability of a battery of immunohistochemical assays that might aid in the proper characterization of well-differentiated prostatic carcinoma (see 4th paragraph on page 384). A malignant index (MI) was developed in their study based on the immunohistochemical staining of six commercially available antisera against cytokeratin (cyto P and cyto M), epithelial membrane antigen (EMA), NK cells (Leu-7), prostatic acid phosphatase (PAP), and prostate specific antigen (PSA). Rubenstein *et al.* concluded that the MI was significantly greater in malignant tissues (e.g. CAP) than in benign tissues (e.g. BPH), suggesting that the MI may be useful as an aid in determining a tissue specimen as benign or malignant. One of the antisera used in Rubenstein *et al.* has reactivities specific for Leu-7. The Leu-7 antigen is an epitope in the MUC18 polypeptide.

Applicant submits that the claimed invention cannot be anticipated by the teachings of Rubenstein *et al.* Anticipating reference must teach each and every element of the claimed invention. MPEP 2131. The claimed invention is a method for predicting an increased risk for metastasis of a prostate cancer cell by comparing the expression levels of MUC18 in a prostate cancer cell and a normal prostate cell. If the level of MUC18 expression is higher in the prostate cancer cell than in the normal prostate cell, the prostate cancer cell has an increased risk of metastasis. Thus, the claimed method is a useful prognostic tool for prostate cancer cells. By contrast, Rubenstein et al. teach a method of using a MI value that is calculated based on the expression of six antigens for distinguishing between CAP (malignant) and BPH (benign). Rubenstein et al. does not teach how to predict an increased risk for metastasis of a prostate cancer cell based on the expression of the MUC18 coding sequence alone.

Liu *et al.* tested the value of the Leu-7 antigen as a prognostic factor for prostate cancer by using the anti-HNK-1 (Leu-7) monoclonal antibody (Mab) and found that the epitope recognized by this Mab is decreased or absent with increasing pathological grades of prostate cancer. Although authors dismissed these results as insignificant, this finding is actually the opposite of the teachings of the claimed invention.

Shih *et al.* describes the Leu-7 antigen as an epitope of the melanoma-associated antigen. There is no mention of prostate cancer in the Shih *et al.*

In summary, based on the foregoing, Applicant submits that the claimed invention is not anticipated by Rubenstein *et al.*, Liu *et al.* or Shih *et al.* Withdrawal of the rejection under 35 U.S.C. § 102 is respectfully requested.

Rejection under 35 U.S.C. § 103:

Claims 2-5 and 7-12 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Rubenstein *et al.* in view of Liu *et al.*, Shih *et al.*, US Patent No. 5,807,978, and US Patent No. 6,057,105. Applicant respectfully traverses this rejection.

The shortcomings of Rubenstein *et al.*, Liu *et al.* and Shih *et al.* have been discussed above. The MI described in Rubenstein *et al.* is based on the use of six different antisera for six antigens. The MI value obtained for a given sample may be useful in distinguishing between a benign and a malignant sample. A person of ordinary skill in the art cannot find suggestion or derive motivation from Rubenstein *et al.* to make the claimed invention. A rejection for obviousness over a combination of references cannot be sustained unless motivation to combine the teachings therein can be found within the references themselves. *In re Jones*, 21 USPQ2d 1941 (Fed. Cir. 1992). The shortcomings of Rubenstein *et al.* are not cured by Liu *et al.*, Shih *et al.*, US Patent No. 5,807,978 or US Patent No. 6,057,105. US Patent 5, 807, 978 describes the immunogenic peptides of prostate specific antigen (PSA). US Patent 6,057,105 describes methods applicable for melanoma and breast cancer cells. There is no teaching or suggestion of MUC18 in conjunction with prostate cancer in either of the cited patents.

In summary, none of the cited references, singly or in combination, provide motivation to one skilled in the art to make and use the claimed invention. Withdrawal of the rejection under 35 U.S.C. 103 is respectfully requested.

CONCLUSION

Based on the foregoing, this case is considered to be in condition for allowance and passage to issuance is respectfully requested.

If there are any outstanding issues related to patentability, the courtesy of a telephone interview is requested, and the Examiner is invited to call to arrange a mutually convenient time.

This Amendment is accompanied by a Request for Continued Examination , a check in the amount of $370 as required under 37 C.F.R. 1.17(e) for a small entity, a Petition for Extension of Time (three months), a check in the amount of $460 as required under 37 C.F.R. 1.17(a)(3) for a small entity, Formal Drawings and Declaration under 37 C.F.R. 1.132. However, if the amount submitted is incorrect, please charge any deficiency or credit any overpayment to Deposit Account No. 07-1969.

Respectfully submitted,

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On page 4, lines 1-5, please replace the first paragraph with the following:

Figs. 2A and 2B [Fig. 2] illustrate[s] Northern blot analysis of expression of human MUC18 in different prostate cancer cell lines. Poly(A)$^+$RNA [Poly(A)+RNA] was isolated from human melanoma cells SK-MEL-28 (SK), human melanocyte (M), and human prostate cancer cells PC-3 (PC-3), DU145 (DU), TSU-PR-1 (TSUPR1), and LNCAP (LNCAP). The size of the human MUC18 mRNA is 3.3 kb. The amount of poly(A)$^+$RNA [poly(A)+RNA] (2.5 to 10 μg) is indicated as a number on top of each lane.

On page 16, line 9 through page 17, line 8, please replace the paragraph bridging pages 16-17 with the following:

Only the middle fragment of the human MUC18 protein can be induced by IPTG to express in a high amount in *E. coli* K-12 strain BL-21. Thus, only this protein is further purified for immunization. When culture A_{600} reaches 0.6 (2 to 3 hours after 1/100 inoculation of an overnight culture in L-broth with ampicillin), the expression of the recombinant middle fragment of MUC18 protein fused to GST in recombinant *E. coli* is induced by addition of 0.1 mM of IPTG to 3-liter cultures (1.5 liters per 4-liter baffled flask). Two hours after addition of IPTG at 37°C, cells are harvested by centrifugation at 3,000 rpm (2,323 x g) for 20 min in a horizontal HG-4L rotor in Sorvall RC-3 centrifuge. The cell pellet is suspended in 40 ml of ice-cold PBS (10 mM Na_2HPO_4 [Na_2HPO4], 1.8 mM KH_2PO_4 [KH_2PO4], 2.7 mM KCl, and 140 mM NaCl, pH 7.3) and then lysed with a prechilled French pressure cell at 800 psi. The lysate is clarified by centrifugation for two to three times at 13,000 rpm (21,000 x g) for 30 min in SS-34 rotor in Sorvall RC-2 centrifuge. The protein concentration of the clear crude lysate adjusted to 10 mg/ml protein (about 60 ml) was used as the starting material for purification. The recombinant MUC18 proteins are purified from the clear crude lysate by batchwise adsorption to the Glutathione-Sepharose 4B affinity resin (about 20 ml of 50% slurry) by inversion on an

inversion shaker at room temperature for 30 min. The GST portion of the fusion protein mediates the binding of the protein to the resin via the glutathione. After twice washing with 10 volumes (50 ml per 5 ml packed resin) of 1 X PBS and followed by twice washing with 1 X PreCission protease cleavage buffer (50 mM TrisHCl, pH 7.0, 150 mM NaCl, 1 mM EDTA, 1 mM DTT) to remove unbound proteins, the fusion protein on the resin is cleaved with 100 units of HRV-3C protease (PreCission protease, 2 units/μl, from Pharmacia) by rocking on an inversion shaker for 16 hours at 4°C. The resin was spun down at 2,000 rpm for 10 min in a Sorvall RC-32 centrifuge. The supernatant and three washings (10 ml 1 X PBS per 10 ml resin), which contain the recombinant MUC18 protein, are then combined and concentrated by centrifuging through a Centricon-30 (Millipore/Amicon). The purity of the protein is characterized by SDS-PAGE (8 to 10% polyacrylamide gel, slab gel). The 70kDa contaminated protein is removed by passing through a Superdex 200 HR 10/30 column in 1 X PBS (void volume about 7 ml for a 20 ml packed column), and the fractions containing the recombinant middle fragment MUC18 protein (22 kDa) (eluted at about 15.5 ml) were pooled. Figs. 2A and 2B [Fig. 2] show[s] the SDS-PAGE results or recombinant huMUC18 protein in the GST-fusion system.

2. (Once amended) The method of claim [1] 20, wherein said prostate cancer cell is from a biopsy tissue sample from a patient for whom a prediction of metastasis of prostate cancer is sought.

4. (Twice amended) The method of claim [3] 20, wherein expression of the MUC18 coding sequence is determined by immunoassay using antibody that recognizes and binds specifically to an epitope of MUC18 wherein said antibody is made in an experimental animal in response to the MUC18 antigen consisting of the amino acid sequence set forth in SEQ ID NO:2.

5. (Twice amended) The method of claim 4, wherein the MUC18 antigen is a middle portion of the MUC18 polypeptide [coding sequence] and [consisting] consists of the amino acid residues 211-376 of the amino acid sequence as set forth [amino acid residues 211-376 of] in SEQ ID NO:2.

20. (Twice amended) A method for [identifying metastatic potential] predicting an increased risk for metastasis of a prostate cancer cell [expressing the gene encoding MUC18] that expresses a MUC18 coding sequence, said method comprising the steps of:

a) measuring the levels of expression of [a] the MUC18 coding sequence in both the prostate cancer cell and a normal prostate cell,

b) comparing the levels of expression of the MUC18 coding sequence in the prostate cancer and normal cells, wherein higher level of expression of the MUC18 coding sequence in the prostate cancer cell relative to the level of expression in the normal prostate cell is positively correlated with [metastatic potential,] an increased risk for metastasis.

[whereby metastatic potential of the prostate cancer cell is deemed high when the level of expression of the MUC18 coding sequence is higher in said prostate cancer cell than in the normal prostate cell.]